

# Second Hematopoietic Stem Cell Transplantation in Pediatric Patients: Overall Survival and Long-term Follow-up

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## ABSTRACT

Despite potent intensive conditioning regimens, hematopoietic stem cell transplantation (HSCT) may fail because of either relapse of the malignancy or the rejection of the graft. We report on 27 pediatric patients who received a second HSCT from an allogeneic donor for relapsed malignancy or graft failure. One-year, 5-year, and 10-year probabilities of survival for all patients were 53%, 36%, and 24%, respectively. Twenty patients received second HSCTs for relapsed malignancy, of whom 6 were alive and disease free at the time of this report. Seven patients received a second HSCT for graft failure, of whom 3 were alive and well as of this report. Twenty-five patients were tested for immune reconstitution following their second HSCT. Sixteen patients developed antigen-specific T-lymphocyte responses; the median time to development of antigen-specific responses was 13 months. There was no significant neurocognitive decline in patients tested 1 to 3 years following their second HSCT. Endocrine evaluations revealed deficiencies in growth hormone (7 patients), gonadal function (3 patients), and thyroid function (2 patients). Three patients developed significant abnormalities of tooth development, including absence of secondary teeth. These results show that a second HSCT offers curative therapy for selected pediatric patients whose first HSCT failed. Although toxicity is considerable following a second transplantation, the major causes of mortality continue to be relapse and infection.

## KEY WORDS

Hematopoietic stem cell transplants • Late effects • Immune reconstitution

## INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative therapy for some hematologic malignancies, immune disorders, and genetic diseases. Despite intensive conditioning regimens, HSCT may fail because of either relapse of the malignancy or rejection of the graft. A second HSCT may be clinically indicated in some patients whose initial HSCT failed.

Approximately 20% to 60% of patients who undergo transplantation for hematologic malignancies relapse after HSCT [1,2]. The management of relapsed patients following HSCT is controversial. The results of chemotherapy alone following HSCT, especially for pediatric acute lymphoblastic leukemia (ALL), have been disappointing, and this treatment rarely results in a cure [3,4]. The results of

newer therapies, such as donor lymphocyte infusions [5,6], have also been disappointing. Previous studies have reported disease-free survival rates of 7% to 38% for patients with ALL following a second HSCT [6-9].

Graft failure occurs most frequently in patients with an intact immune system who receive hematopoietic stem cells (HSC) from either unrelated bone marrow or cord blood. Primary or secondary graft failure has occurred in 14% of recipients of unrelated HSCT [10]. Furthermore, approximately 50% of recipients of unrelated cord blood transplants who have an intact immune system do not have sustained donor hematopoiesis [11]. Previous reports have shown a 17% to 44% disease-free survival rate for patients receiving a second HSCT for graft failure. Most series have studied patients with intact immune systems, such as those

with severe aplastic anemia and thalassemia. Grandage et al. examined 12 patients with graft failure following first HSCT. Two thirds of these patients initially underwent transplantation for leukemia. Of these 12 patients, 5 were alive with a median survival of 38 months; however, only 2 of these patients remained disease free [12-14].

We report on 27 pediatric patients who suffered either relapse or graft failure after their first HSCT and received a second transplant from an allogeneic donor. We describe their clinical outcomes in terms of immune reconstitution, endocrine dysfunction, skeletal deformities, neurocognitive function, and secondary malignancies. Nine of the 27 patients were alive and well at the time of this report, suggesting that a second HSCT is indicated in selected pediatric patients whose first HSCT fails.

## PATIENTS AND METHODS

### Informed Consent

The Committee on Clinical Investigation (Institutional Review Board) of Childrens Hospital Los Angeles reviewed and approved the protocols for initial HSCT, second HSCT, and long-term follow-up studies. All patients were recruited for long-term follow-up studies after informed consent was obtained for their participation in the respective HSCT studies.

### Patients

Between September 1985 and January 2001, 27 pediatric patients with ALL (n = 11), acute myeloblastic leukemia (AML, n = 3), severe combined immunodeficiency (SCID, n = 3), natural killer (NK) cell leukemia (n = 2), myelodysplastic syndrome (MDS, n = 2), juvenile monomyelogenous leukemia (JMML, n = 2), chronic myelogenous leukemia (CML, n = 1), non-Hodgkin's lymphoma (NHL, n = 1), pure red cell aplasia (n = 1), or hemophagocytic lymphohistiocytosis (HLH, n = 1) received second HSCTs. Nineteen patients were boys and 8 were girls. Patient characteristics are shown in Table 1. Patients are identified by their unique patient number (UPN).

Twenty patients received second HSCT for relapse of hematologic malignancy. For these patients, second transplantations occurred between 4 months and 6 years (median, 13 months) following their first HSCT. These 20 patients achieved remission prior to receiving their second HSCT. Seven patients received second HSCT for graft failure, either primary or secondary. Second transplantations for graft failure were performed between 5 weeks and 17 months (median, 3 months) following the first transplantation.

The median age of all recipients at the time of their first HSCT was 4 years (range, 4 months to 15 years), and their median age at the time of their second HSCT was 6 years (range, 8 months to 15 years).

### Pre-HSCT Conditioning Regimens

Preparative regimens and donors for HSCT for the first and second HSCT are shown in Tables 1 and 2. Eighteen patients received related histocompatible HSCTs for both their first and second HSCT. Of the remaining 9 patients, 7 patients received HSCT from alternative donors (unrelated or haploidentical) for both their first and second HSCTs;

4 patients received the same alternative donor product; 2 patients received an autologous HSCT followed by a matched unrelated donor (MUD) HSCT; 2 patients received an unrelated peripheral blood stem cell transplant from the same donor for their second HSCT; and 1 patient received a haploidentical HSCT following an unrelated cord blood transplantation (UCBT). For their first HSCT, 22 patients received busulfan, 16 mg/kg or, for those younger than 5 years, 640 mg/m<sup>2</sup>, and cyclophosphamide, 200 mg/kg. The remaining 5 patients received alternative regimens shown in Table 2. For their second transplantation, 20 patients received total body irradiation (TBI) (1200 cGy, 200 Gy twice a day for 3 days) and etoposide (VP-16), 60 mg/kg. The remaining patients received alternative preparative regimens shown in Table 2.

Graft-versus-host disease (GVHD) prophylaxis consisted of methotrexate (MTX), 10 mg/m<sup>2</sup> on days +3, +6, +11, and +18. Cyclosporin (CSA) was also given to those patients who were older than 10 years and/or whose donors were older than 10 years. Recipients of unrelated HSCT received antithymocyte globulin (ATG) for 7 doses, on alternate days, beginning on day +5. Two patients, both of whom had SCID, received T-cell-depleted haploidentical HSCT followed by ATG and methylprednisolone. For the second HSCT, 26 patients received GVHD prophylaxis with CSA and MTX. One patient (UPN 447) received MTX only for GVHD prophylaxis during the second transplantation. Patients who developed GVHD received corticosteroids as first-line GVHD therapy. Patients who remained on CSA also received monthly intravenous immune globulin (IVIG) replacement therapy.

### Immune Reconstitution

Both cellular and humoral immunological functions were determined following the second HSCT. Patient immune function was evaluated by determining the antigen-specific T-lymphocyte blastogenesis following specific antigenic stimulation (tetanus toxoid, *Candida*, cytomegalovirus [CMV], herpes simplex virus [HSV], and varicella zoster virus [VZV]) as previously described [15]. Positive responses were defined as a  $\Delta\text{cpm} \geq 3000$ . Antibodies to polyribose phosphate (PRP) were measured by an enzyme-linked immunosorbent assay (ELISA) as previously described [16].

### Long-term Follow-up

Patients who survived more than 1 year following their HSCT and remained in remission were evaluated in a long-term follow-up study. Patients who underwent transplantation prior to 1993 did not have pre-HSCT testing performed and were therefore not eligible for the present analysis. Patients who were not being treated with clonidine for hypertension had assessment of the growth hormone axis with clonidine stimulation. Abnormal responses were defined as <10 ng/mL after oral clonidine. Levels of free thyroxine, thyroid-stimulating hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol or testosterone were measured to assess thyroid and gonadal function. Elevated LH and FSH levels for age were considered evidence of primary gonadal failure. Patients were routinely assessed for dental abnormalities and the presence of avascular necrosis of the bone (AVN).

Table 1. Patient Characteristics\*

Patient UPN	Age†	Sex	Diagnosis	Cause of Second HSCT	Time Between HSCTs	Conditioning Regimen of First HSCT	Donor for First HSCT	Conditioning Regimen of Second HSCT	Donor for Second HSCT	Status
11	5 y	M	Pure red cell aplasia	Primary graft failure	2 mo	Bu/Cy/TLI/procarbazine/ATG	6/6 sibling	Bu/Cy/TLI/procarbazine/ATG	Same	Alive and well
29	9 y	M	NHL	Relapse	4 mo	TBI/VP-16/ARA-C	6/6 sibling	TBI/VP-16/ARA-C	Same	Died, PD
103	6 y	M	ALL CR2	Relapse	6 y	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, GVHD
184	7 y	F	ALL CR2	Relapse	6 y	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, sepsis/GVHD
188	9 mo	F	Infant ALL	Relapse	8 mo	Bu/Cy	6/6 MUD (BM)	TBI/VP-16	Same	Died, second tumor
214	3 y	M	Mixed lineage ALL	Relapse	26 mo	Bu/Cy	Auto	TBI/VP-16	6/6 MUD (BM)	Alive and well
250	7 y	M	MDS mono-somy 7	Relapse	36 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, GVHD
274	4 y	F	AML	Relapse	32 mo	Bu/Cy	Auto	TBI/VP-16	6/6 MUD (BM)	Alive and well
334	13 y	F	NK cell leukemia	Relapse	12 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Alive and well
364	10 y	F	CML	Relapse	10 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Alive and well
382	9 mo	F	Bare Lymphocyte Syndrome	Primary graft failure	6 mo	Bu/Cy/ATG	Haplo-identical (mother)	Bu/Cy/ATG	Same	Died, graft failure
386	12 y	M	AML CR2	Relapse	7 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Alive and well
399	18 mo	M	JMML	Primary graft failure	3 mo	Bu/Cy/ATG	6/6 MUD (BM)	TBI/VP-16	Same	Died, PD
402	10 y	M	T-ALL CR2	Relapse	20 mo	Bu/Cy	6/6 sibling	TBI/VP-16/ARA-C	Same	Died, PD
411	14 mo	M	HLH	Secondary graft failure	18 mo	Bu/Cy/VP-16/ATG	6/6 MUD (BM)	TBI (sparing head and neck)/Cy	Same	Alive and well
434	2 y	F	SCID/NHL	Primary graft failure	1 mo	Cy/ATG	6/6 mother	TBI/VP-16/Cy/ARA-C	Same	Died, PD
447	3 y	M	AML CR2	Relapse	4 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
450	16 mo	M	ALL, mono-somy 7	Relapse	13 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
453	8 y	M	ALL CR2	Relapse	10 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Alive and well
461	18 mo	M	JMML	Relapse	19 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
464	9 y	F	ALL CR2	Relapse	16 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
477	4 y	M	Ph+ ALL	Relapse	4 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
308	5½ y	F	ALL CR2	Relapse	10 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, PD
562	5 mo	M	SCID	Primary graft failure	3 mo	Bu/Cy/ATG	4/6 UCBT	Bu/Cy/ATG	Haplo-identical (mother)	Alive and well
622	3 y	M	MDS	Relapse	8 mo	Bu/Cy/ATG	6/6 MUD (BM)	TBI/VP-16	Same donor PBSC	Died, PD
660	15 y	M	NK cell leukemia	Primary graft failure	2 mo	TBI/VP-16	6/6 MUD (BM)	Fludarabine/Cy/TLI	Same donor PBSC	Died, sepsis/organ toxicity
561	10½ y	M	ALL CR2	Relapse	27 mo	Bu/Cy	6/6 sibling	TBI/VP-16	Same	Died, GVHD

\*Bu indicates busulfan; Cy, cyclophosphamide; TLI, total lymphoid irradiation; ARA-C, cytarabine; PD, progressive disease; CR, complete remission; Auto, autologous; Ph, Philadelphia chromosome; PBSC, peripheral blood stem cells.

†Age at first HSCT.

### Neurocognitive Function

Prior to their first HSCT, most patients were evaluated to determine their neurocognitive function. One to 2 years following their second HSCT, patients were reevaluated.

Tests were administered in the patient's primary language, either English or Spanish. Domains that were evaluated included overall cognitive/intellectual functioning, verbal skills, performance skills, and receptive vocabulary.

**Table 2.** Transplantation-Related Data

	HSCT No. 1, n	HSCT No. 2, n
<b>Donor</b>		
Autologous	2	0
Histocompatible	18	18
Haploidentical	1	2
Matched unrelated BM	5	5
Matched unrelated PBSC	0	2
Matched unrelated cord blood	1	0
<b>Preparative regimen</b>		
Bu/Cy	22	1
Bu/Cy/VP-16	1	0
Bu/Cy/procarbazine/TLI	1	1
Bu/Cy/TBI	0	1
TBI/VP-16	1	19
TBI/Cy	0	1
TBI/VP-16/ARA-C	1	2
TBI/VP-16/Cy/ARA-C	0	1
Cy only	1	0
Fludarabine/Cy/TLI	0	1

### Statistical Analysis

The probability of event-free survival was calculated by Kaplan-Meier analysis [17]. Data on patients were censored at the first adverse event: death due to treatment-related toxicity, graft failure, relapse, or other causes. Paired Student *t* tests were used for statistical analysis of the neurocognitive tests.

## RESULTS

### Outcome of HSCT for the Primary Disease

Kaplan-Meier analysis of the probability of event-free survival following a second HSCT is shown for patients with either relapsed malignancies or graft failure (Figure 1). One-year, 5-year, and 10-year probabilities of survival for all patients were 53%, 36%, and 24%, respectively. At the time of this report, 9 of the 27 patients who received a second HSCT were alive and disease free and 6 of the 20 patients who received a second HSCT for relapsed malignancy were alive and disease free. Causes of death for the study patients were relapse (9 patients), sepsis/toxicity (2 patients), acute GVHD (2 patients), and secondary malignancy (1 patient).

Of the 7 patients who received a second HSCT for graft failure, 3 (43%) were alive as of this report. Causes of death for the other 4 patients included relapse (2), sepsis (1), and graft failure (1).

Eleven patients developed clinically detectable GVHD. Five patients had grade 3 to 4 acute GVHD, and 6 patients developed chronic GVHD. First-line therapy for acute GVHD was corticosteroids. Patients who developed steroid-nonresponsive acute GVHD required intensive immunosuppressive therapy with other agents, including ATG, tacrolimus, and daclizumab.

### Immune Reconstitution

The immune reconstitution of 25 patients was longitudinally evaluated following their second HSCT. The remaining 2 patients died too early to be evaluated. Of the 25 patients tested, 16 (64%) developed antigen-specific responses following HSCT. The median time to the devel-

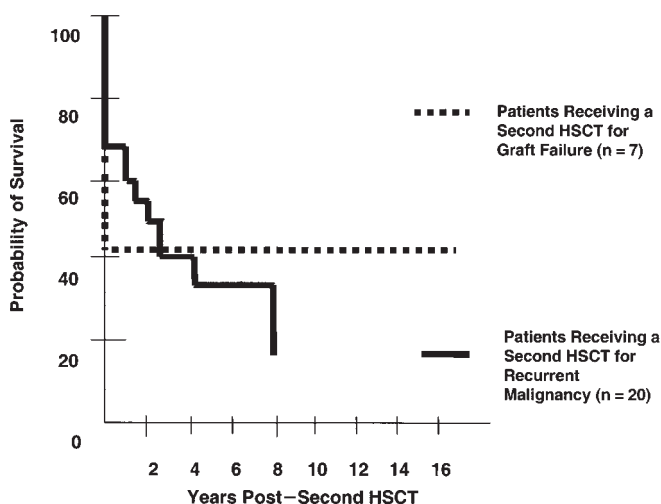
opment of their first antigen-specific response was 13 months (range, 3-34 months). The remaining 9 patients did not develop antigen-specific T-lymphocyte proliferative responses within follow-up periods of 34 months or less.

Following their second HSCT, 16 patients were evaluated for their response to a naturally occurring bacterial carbohydrate, Polyribose phosphate (PRP) the capsular antigen of *Hemophilus influenzae*. PRP testing was not performed on patients receiving IVIG therapy. Because patients receiving CSA remained on IVIG, these patients were not evaluated for their response to PRP. Only 3 patients had normal PRP responses (defined as a value >100 ng/mL). Eight patients developed subnormal PRP responses (15-100 ng/mL) at an average of 21 months (range, 3-46 months) following their second HSCT. Eight patients did not develop any PRP antibody response following their second HSCT. The 3 patients who had normal PRP levels all received histocompatible HSCT from a HLA-matched sibling. None of the patients who received a MUD HSCT developed normal PRP antibody responses.

### Endocrine Function

The patients who survived more than 1 year following their second HSCT and were on minimal or no steroid therapy received long-term endocrine evaluations. The results are summarized in Table 3. The most significant abnormalities were growth hormone (GH) deficiency (7 of 11 patients tested), primary gonadal insufficiency (3 of 6 patients), and hypothyroidism, either primary or central (2 of 11 patients).

Of the 7 patients who developed GH deficiency, 2 patients were receiving GH replacement as of this report. One patient died prior to initiation of GH replacement therapy. Of the remaining 4 patients, 3 patients remained below the mean for height but were within 2 standard deviations for age as of this report. The fourth patient (UPN 411) was too young to begin GH therapy. One patient (UPN 188) began GH replacement but developed an osteosarcoma within 1 year.



**Figure 1.** Kaplan-Meier probability of disease-free survival for patients receiving a second HSCT for graft failure (— · — · —) and relapsed malignancy (————).



**Table 3.** Results of Long-term Follow-up

	No. Affected*	No. Tested
<b>GH</b>	<b>7</b>	<b>11</b>
<b>Gonadal function</b>		
Ovarian	2	6
Testicular	1	
<b>Thyroid</b>	2	11
<b>GVHD</b>		
Skin	11	
Gastrointestinal	4	
Lungs	4	
Liver	1	
<b>AVN</b>	2	
<b>Abnormal tooth development</b>	3	
<b>Secondary malignancy</b>	1	

\*Of the 27 patients who received a second HSCT.

Three patients had evidence of primary gonadal dysfunction (ovarian 2, testicular 1). The 2 female patients with ovarian insufficiency (UPN 334 and 364) received their first HSCT at the ages of 13 years and 10 years, respectively, and their second HSCT at the ages of 14 and 11 years, respectively. The male patient with testicular insufficiency (UPN 214) had his first HSCT at the age of 3 years and his second HSCT at the age of 5 years. All 3 patients received busulfan/cytosine as their first conditioning regimen and TBI/VP-16 for their second HSCT. As of this report, the female patients remained on estrogen/progestin replacement, and the male patient was receiving GH therapy prior to the planned institution of testosterone replacement.

Two patients developed central hypothyroidism. One patient (UPN 214) was receiving thyroid hormone replacement as of this report, and the remaining patient (UPN 103) died of pulmonary complications.

One patient (UPN 274) developed insulin-dependent diabetes mellitus 6 years after her second HSCT. Her post-transplantation course was complicated by mild skin GVHD. Her other complications included GH deficiency and hypothyroidism. There is no known family history of diabetes, and her MUD donor's diabetes status is unknown.

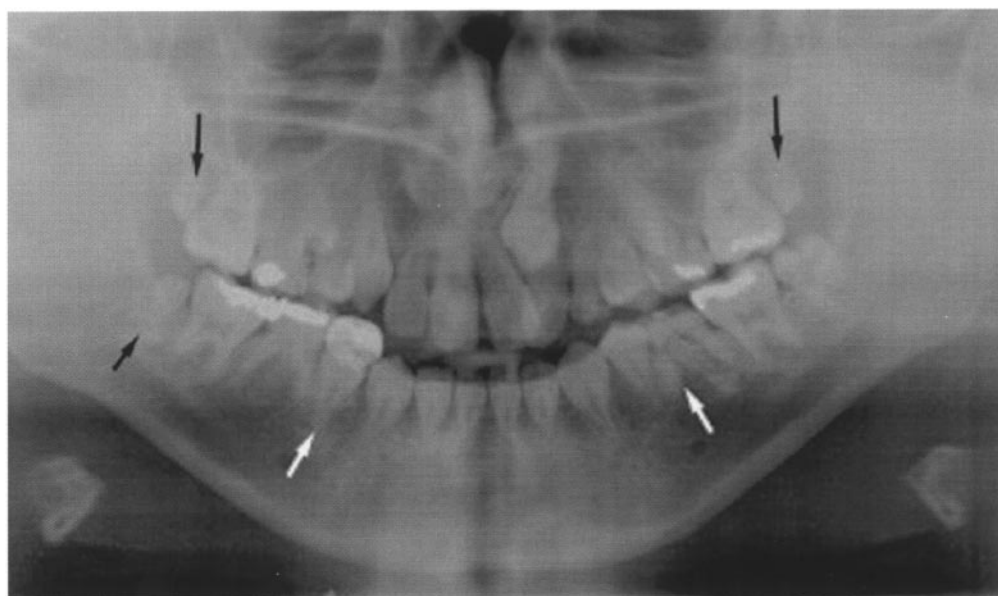
### Skeletal and Dental

Two patients (UPN 334 and 364) who received related histocompatible second HSCT developed AVN of the hips and required subsequent unilateral hip replacements. Both of these patients had significant GVHD and received prolonged steroid therapy. GVHD resolved in both patients, and steroid therapy was discontinued. However, both patients continued to have pain in other large joints.

Three patients (UPN 188, 214, and 411) had severe dental abnormalities, including absence of secondary teeth and microdontia. The dental x-ray of 1 patient (UPN 214) is shown in Figure 2. UPN 214 and 411 were undergoing evaluation for dental implants and restorations as of this report. UPN 188 had minimal dental restorations prior to developing a secondary malignancy.

### Neurocognitive Function

Neurocognitive functioning was evaluated prior to the first HSCT and at 1 to 3 years following the second HSCT. Pre-HSCT evaluations were performed on patients within 1 month of hospital admission for HSCT. The overall neurocognitive function mean score was 87 (pre-initial HSCT) versus 72.8 (post-second HSCT) ( $P = .13$ ,  $n = 6$ ). When pre-initial-HSCT scores were compared to the post-second-HSCT scores, there were also decreases in both mean verbal and performance skills scores; however, these decreases were not significant (verbal skills decreased from 96.5 [pre] to 89.75 [post],  $P = .57$ ,  $n = 4$ ; performance scores decreased



**Figure 2.** Dental panoramic x-ray of patient UPN 214 obtained 7 years post-second HSCT. The black arrows point to the areas of microdontia, the bilateral upper second molars, and the lower right second molar. The white arrows represent absent secondary teeth at the lower second premolar (the bicuspid) and the third molar.



**Figure 3.** X-ray from patient UPN 188. Six years following her second HSCT, this patient developed pain and swelling. The arrow indicates the osteosarcoma at the right distal femur. The osteosarcoma was most likely the result of the radiation therapy she received for her second HSCT.

from 88.4 [pre] to 84.2 [post],  $P = .69$ ,  $n = 5$ ). In contrast, receptive vocabulary scores increased from 89.3 to 99.3 ( $P = .46$ ,  $n = 3$ ). The neurocognitive tests are age based; therefore because of their age some children did not receive specific tests. Consequently, the numbers of patients tested in each of the neurocognitive domains differed.

### Secondary Malignancy

Patient UPN 188 developed a secondary malignancy (osteosarcoma) 6 years following her second transplantation for infantile ALL. This patient received her first HSCT at the age of 9 months and the second HSCT at the age of 17 months (Figure 3). Her conditioning regimen for the first HSCT consisted of busulfan and cytoxan and that for her second HSCT consisted of TBI (1200 Gy) and VP-16 (60 mg/kg). She developed a secondary malignancy 1 year after initiation of GH replacement therapy. She died of progressive osteosarcoma.

### DISCUSSION

Relapse and graft failure following HSCT are devastating complications with high mortality rates. Our analysis of

27 pediatric patients who underwent second HSCTs shows that the probability of 10-year event-free survival is 24%. The majority of our patients received a non-TBI-based regimen for their first HSCT. Previous studies have shown similar survival rates for patients receiving TBI-based regimens for their first HSCT [7,8]. It might be expected that a longer interval between first and second HSCT might result in a better outcome because patients would then have a longer period to recover from the toxic effects of the first HSCT. Seventeen patients with relapsed leukemia received their second HSCT more than 6 months following their first HSCT; 7 of these patients were alive and disease free as of this report. Of the patients who received their second HSCT for relapsed malignancy less than 6 months following their first HSCT, 1 of 3 remained alive and disease free. These numbers are too small to be the basis of any meaningful conclusions.

In our series of pediatric patients receiving second transplantations for graft failure, at the time of this report 3 of 7 patients were alive following their second HSCT. Of note, UPN 562 developed graft failure following HSCT with unrelated cord blood that was later found to have been obtained from a donor with a chromosomal abnormality (XXY). This patient subsequently underwent another transplantation with a haploidentical HSCT and was alive and well as of this report [18]. Overall, our results are comparable to previous results of second transplantations for graft failure.

It might be expected that the recipients of 2 HSCT would have more transplantation-related side effects than would the recipients of only 1 HSCT. Eleven of the second-HSCT recipients developed acute and/or chronic GVHD. Five patients developed severe (grade 3 or 4) acute GVHD. Sixteen patients did not develop any GVHD with either HSCT. Thus, a second HSCT does not increase the likelihood of developing GVHD.

GVHD and its therapies are associated with immune deficiency in HSCT recipients. Recipients of HSCT are at increased risk of bacterial and viral infections, suggesting defects in both humoral and cellular immunity. Weinberg et al. have reported that GVHD is the major cause of defective thymopoiesis after HSCT. Cytotoxic therapy may adversely impact thymopoietic function [19-21]. We evaluated the impact of a second myeloablative conditioning regimen on recipient immune function. Sixteen (67%) of 24 patients developed antigen-specific responses at a median of 13 months from their second HSCT, whereas 8 patients did not develop any antigen-specific responses within a maximum follow-up period of 34 months. Other investigators have shown that a T-lymphocyte proliferative response to HSV can be detected in HSV-positive HSCT recipients as early as 40 days following HSCT and that this response is followed by the acquisition of responses to VZV and CMV [22]. Routine acyclovir is known to delay the appearance of T-lymphocyte proliferative responses by inhibiting viral reactivation [22,23]. Because all of the herpes virus-seropositive patients received prophylactic acyclovir following their second HSCT, the time to acquire T-lymphocyte proliferative responses to herpes virus would be expected to be delayed. The kinetics of cellular reconstitution after a second HSCT did not differ from that seen following a first HSCT.

Kapoor et al. have previously shown that unrelated HSCT recipients and a significant proportion of histocompatible

HSCT recipients have prolonged defects in their capacity to produce antibodies to bacterial carbohydrate antigens [24]. In the present series, only 3 patients, all of whom received histocompatible HSCT, developed normal PRP responses following their second HSCT. Therefore, the recipients of second HSCTs, like all HSCT recipients, need to be routinely monitored for their immune reconstitution and should remain on prophylactic antibiotics and IVIG until adequate anticarbohydrate antibody function is present.

Chemotherapy, irradiation, GVHD, and infection may all contribute to long-term toxicities (endocrine, skeletal, and neurocognitive) in recipients of HSCT. Recipients of a second HSCT might be at an increased risk of endocrine complications (impaired growth, ovarian or testicular dysfunction, abnormal pubertal progression, hypothyroidism, and panhypopituitarism) because they have received multiple agents that can affect the hypothalamic/pituitary axis, thyroid gland, and gonads (TBI, MTX, and steroids) [25,26]. In our series, 4 patients had multiple endocrinopathies (GH, gonad, thyroid, and diabetes). The presence of multiple endocrinopathies in 4 of our patients leads us to suspect that recipients of 2 HSCTs are at increased risk of developing multiple endocrinopathies compared to recipients of a single HSCT.

Gonadal dysfunction in pediatric transplantation patients is thought to be rarer than in adult patients because prepubertal gonads are quiescent. However, many pediatric patients treated with chemotherapy show evidence of hormonal dysfunction as they mature. Sanders et al. reported that patients who have received HSCT with either high-dose cyclophosphamide or TBI-based regimens are at increased risk of gonadal dysfunction [25]. In our series, 11 patients were studied for gonadal dysfunction; 2 patients had ovarian dysfunction, and 1 had testicular dysfunction. These results should be interpreted with some caution because many of the surviving patients were young and prepubescent. As the age of the survivors increases, the frequency of gonadal dysfunction may increase.

Children who receive a significant amount of irradiation are at risk of developing thyroid dysfunction. Approximately 10% of childhood ALL survivors who received both chemotherapy and 18 to 24 cGy of craniospinal irradiation develop hypothyroidism [27]. Because the TBI dose used for HSCT was 12 cGy, most of our second-HSCT recipients retained relatively normal thyroid function.

Young children who receive irradiation are known to be at risk for hypoplasia of their developing teeth [28,29]. Patients who are not HSCT recipients and are treated with chemotherapy alone do not appear to have severe dental complications. A review of more than 700 patients who received a single HSCT at the Childrens Hospital Los Angeles identified only a single patient who had absent secondary teeth; the patient had infantile ALL and had received cranial irradiation at 2 years of age prior to HSCT. In the present series, 3 patients who received second HSCT developed significant dental abnormalities. Two patients (UPN 188 and 411) received their first HSCT during infancy (<12 months), whereas the third patient (UPN 214) received his first HSCT at 3 years of age (Figure 3). Although cranial irradiation seems to be the strongest factor related to dental abnormalities, 1 of our patients (UPN 411) with significant dental

abnormalities did not receive cranial irradiation during either HSCT, suggesting that children who receive 2 HSCT should be followed closely for abnormal dental development.

The majority of previous studies have shown that HSCT has a negative impact on neurocognitive function [30,31]. In some studies there has been no significant decline in neurocognitive functioning 1 to 3 years following HSCT [32]. We studied the impact of 2 HSCTs on neurocognitive function. Although the raw neurocognitive test scores show decreased neurocognitive function, there were no statistically significant differences when the pre-first-HSCT and the post-second-HSCT (1-3 years) results were compared. With longer follow-up, however, definable differences may be seen.

Of all of the possible late effects of chemoradiotherapy, the risk of developing a second cancer is one of the most severe. An estimated 3% to 12% of children treated for cancer will develop a secondary malignancy within 20 years from their first diagnosis. Exposures to radiation therapy and chemotherapy (mainly alkylating agents) are known risk factors for developing secondary cancers. In our cohort, only 1 patient developed a secondary cancer, an osteosarcoma 6 years post-second HSCT. However, the number of long-term survivors is low, and patients must continue to be monitored for the development of secondary malignancies.

In conclusion, second HSCT can be successfully performed for selected pediatric patients who suffer relapse or graft failure following their initial HSCT. The major causes of mortality continue to be relapse and infection. Although there are long-term complications following a single HSCT, some of these complications appear to be more severe following 2 HSCT (abnormal dental development and multiple endocrinopathies). Younger patients are susceptible to unique toxicities that differ from those occurring in adult patients and need to be longitudinally evaluated for these complications.

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